(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) EP 1 148 119 A1

(12) EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
24.10.2001 Bulletin 2001/43

(21) Application number: **00976258.4**

(22) Date of filing: **15.11.2000**

(51) Int Cl.[7]: **C12M 1/00**, C12M 1/42, G01N 33/53, G01N 33/566, G01N 33/532, G01N 21/76, C12Q 1/68

(86) International application number:
PCT/JP00/08049

(87) International publication number:
WO 01/38482 (31.05.2001 Gazette 2001/22)

(84) Designated Contracting States:
AT BE CH CY DE DK ES FI FR GB GR IE IT LI LU MC NL PT SE TR

(30) Priority: **25.11.1999 JP 33412099**

(71) Applicant: **Hitachi Software Engineering Co., Ltd.**
Yokohama-shi, Kanagawa 231-8475 (JP)

(72) Inventors:
- **TANAKA, Toshiaki,**
  Hitachi Software Eng. Co.,Ltd
  Yokohama-shi, Kanagawa 231-8475 (JP)
- **YAMAMOTO, Kenji,**
  Hitachi Software Eng. Co.,Ltd
  Yokohama-shi, Kanagawa 231-8475 (JP)
- **HATANO, Koichiro,**
  Hitachi Software Eng. Co.,Ltd
  Yokohama-shi, Kanagawa 231-8475 (JP)
- **MIZUNO, Katsuya, Hitachi Software Eng. Co., Ltd**
  Yokohama-shi, Kanagawa 231-8475 (JP)

(74) Representative: **Liesegang, Roland, Dr.-Ing. et al**
FORRESTER & BOEHMERT
Pettenkoferstrasse 20-22
80336 München (DE)

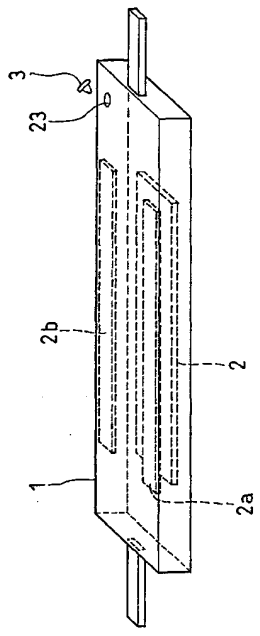
(54) HYBRIDIZATION DEVICE, CASE, SUPPORT, AND LABEL AGENT

(57) The present invention provides a hybridization device, case, support and labeling reagent which can enhance the efficiency of the hybridization reaction, save more time for the reaction and improve the detection sensitivity.

A case **(1)** comprises: a metal support **(2)** made of platinum-covered titanium and having probe DNA immobilized thereon; counter electrodes **(2a)** and **(2b)** for applying a voltage between the metal support **(2);** a cap **(3);** and a filler port **(23).**

As a result, a hybridization reaction can be performed in an efficient manner in a short time. In addition an electrogenerated chemiluminescent substance may be used for detection.

Fig. 1



EP 1 148 119 A1

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a hybridization device, a case for performing a hybridization reaction in the device, a support for performing a hybridization reaction in the case, and a labeling reagent for labeling a biological substance used for the hybridization.

BACKGROUND ART

[0002] Conventionally, a hybridization reaction include steps of: immobilizing probes on an insulating glass plate as a support; dropping a hybridization reaction solution containing a fluorescence-labeled sample on the support; covering the support with a glass cover; and leaving the support in a thermostat for a predetermined period of time. Thereafter, the support is taken out from the thermostat, and washed with a washing solution. The fluorescent substance as the label is excited and the resulting fluorescence is read with a detector, thereby identifying the sample hybridizing to the probes. The above-mentioned probes and sample are all biological substances, specifically DNA or RNA. Hybridization may take place between DNA and RNA. Alternatively, samples may be immobilized on a support to be subjected to hybridization with a hybridization reaction solution containing a fluorescence-labeled probe. Herein, a hybridization reaction between DNA probes immobilized on a support and labeled DNA are described as an example. However, the present invention is not limited thereto.

[0003] When a hybridization reaction is carried out as described above, the reaction takes 6 to 7 hours, requiring long time. Thus, in general, multiple expensive hybridization devices are used in line to perform multiple hybridization reactions at the same time, which requires a large installation space.

[0004] The present invention has an objective of providing a hybridization device, case, support and labeling reagent, which can enhance the efficiency of a hybridization reaction, save more time for the reaction and improve the detection sensitivity.

DISCLOSURE OF THE INVENTION

[0005] A support of the present invention comprises a metal for supplying a charge to a hybridization reaction solution. Accordingly, a charge can be supplied to the reaction solution to attract a biological substance contained in the hybridization reaction solution to the support side. Moreover, an electrogenerated chemiluminescent substance may be used as a labeling reagent.

[0006] By immobilizing a biological substance on the support, the support may, for example, be used directly for diagnosis of a specific disease.

[0007] A case of the present invention comprises an electrode for supplying a charge to a hybridization reaction solution.

[0008] By storing the above-described support, the case may, for example, be used directly for diagnosis of a specific disease as described above.

[0009] A hybridization device of the present invention supplies electricity to a case used for a hybridization reaction to supply a charge to a hybridization reaction solution.

[0010] A labeling reagent of the present invention comprises an electrogenerated chemiluminescent substance for labeling a biological substance.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011]

Figure **1** is a perspective view showing a structure of a case used for a hybridization reaction according to one embodiment of the present invention;

Figure **2** is a schematic view showing a structure of a hybridization device according to one embodiment of the present invention;

Figure **3** is a schematic view for illustrating the hybridization reaction according to one embodiment of the present invention;

Figure **4** is a schematic view for illustrating a structure of sample DNA introduced with a ruthenium complex according to one embodiment of the present invention;

Figure **5** is a schematic view showing electrogenerated chemiluminescence according to one embodiment of the present invention;

Figure **6** is a diagram (part 1) for illustrating a reaction between the ruthenium complex and TPA on a metal support; and

Figure **7** is a diagram (part 2) for illustrating the reaction between the ruthenium complex and the TPA on the metal support.

BEST MODES FOR CARRYING OUT THE INVENTION

[0012] Hereinafter, a preferred embodiment of the present invention will be described in detail with reference to the accompanying drawings.

[0013] Figure **1** is a perspective view showing a structure of a case used for a hybridization reaction according to one embodiment of the invention.

[0014] The case **1** is provided with a metal support **2,** counter electrodes **2a** and **2b,** a cap **3** and a filler port **23.**

[0015] Preferably, the case **1** is transparent for optically detecting the results of the reactions and is made of an acrylic resin to be resistant against chemicals. The metal support **2** is firmly attached to the center of the inner bottom of the case **1.** The counter electrodes **2a** and **2b** are firmly attached to the case **1** above the both sides of the metal support **2** so that the counter electrodes **2a** and **2b** do not interfere with observation from

above.

[0016] The metal support **2** has a size of, for example, 25 x 75 x 2 mm^3 and is immobilized with probe DNA. Accordingly, the surface of the metal support **2** has to be uniform and needs to be stable as an electrode. Therefore, in this embodiment, platinum-covered titanium is used.

[0017] Since the counter electrodes **2a** and **2b** are not transparent in this case, they have to be placed such that they do not interfere with the detection with a cooled CCD. If a transparent electrode is used in place of the counter electrodes **2a** and **2b,** a single electrode as large as the metal support **2** may be provided above the metal support **2.**

[0018] Figure **2** is a schematic view showing a hybridization device according to the embodiment of the invention. The case **1** is mounted with the metal support **2,** injected with a hybridization reaction solution, and is sealed with the cap **3.** The case **1** is then placed on and heated by a Peltier element **4.** The Pettier element **4** is connected to a computer **13** so that the reaction temperature can be controlled. The metal support **2** is charged positive by a power switch **5** and a voltage is applied between the metal support **2** and the respective counter electrodes **2a** and **2b,** thereby applying an electric field to the reaction hybridization solution for a hybridization reaction. The power switch **5** is connected to and controlled by the computer **13.** The voltage applied for this hybridization reaction is about 100 V. After the reaction, the hybridization reaction solution is discharged from the case **1** to a discharged solution reservoir **8** via a discharging tube **6.** By doing so, un-reacted sample DNA **16** (see Figure **3)** is discharged along with the hybridization reaction solution. Thereafter, a washing solution in a washing solution reservoir **9** is injected into the case **1** via an injection tube **7** by the pump **14,** and similarly discharged into the discharged solution reservoir **8.** According to the present embodiment, 0.2 x SSC/0.1% SDS solution is used as the washing solution. Furthermore, a TPA (Tripropylamine) solution in a TPA solution reservoir **10,** which is necessary for the later-described electrogenerated chemiluminescence is injected into the case **1.** The solution to be injected is selected with a switch **15.** After injecting the TPA solution, the power switch **5** is turned on again to apply a voltage to the metal support **2** for electrogenerated chemiluminescence. The current for the electrogenerated chemiluminescence is about 100 μA. However, since this current is required for oxidizing later-described Ru^{2+} to obtain Ru^{3+}, an optimal luminescence intensity is adjusted at about 50 to 150 μA (variable). The luminescence is detected by the cooled CCD **11** on the case **1.** After the detection, the TPA solution in the case **1** is discharged. The detected data is sent to the computer **13** via an A/D converter **12.**

[0019] Figure **3** is a schematic view for illustrating the hybridization reaction according to the embodiment of the present invention. The power switch **5** is turned on to apply a voltage so that the negatively-charged sample DNA **16** in the case **1** is attracted to the positively-charged metal support 2, thereby increasing the chance of the reaction and thus improving the reaction efficiency of the reaction with the probe DNA on the metal support **2.** As a result, time required for the hybridization reaction can be saved.

[0020] During the reaction, the case **1** is heated from beneath by the Peltier element **4** to maintain a constant reaction temperature.

[0021] Figure **4** is a schematic view for illustrating a structure of the sample DNA **16** introduced with a ruthenium complex **18.** The sample DNA **16** in the hybridization reaction solution is modified with an electrogenerated chemiluminescent substance. The present device employs the ruthenium complex **18** as a luminescent substance and Tripropylamine (TPA) **17** as an electron donor, thereby enabling detection based on electrogenerated chemiluminescence. According to the present embodiment, N-hydroxysuccinimide-activated ester (NHS ester) **19** is used as a cross-linking agent. The NHA ester **19** is bound to streptoavidin **20.** On the other hand, sample DNA **22** is biotinylated with biotin **21.** Through this streptoavidin-biotin binding, the ruthenium complex **18** can be bound to the sample DNA **22.** The sample DNA **22** may be biotinylated by using a biotinylation kit commercially available from Pierce or else.

[0022] Figure **5** is a schematic view for illustrating electrogenerated chemiluminescence according to the embodiment of the present invention. The ruthenium complex 18 modifying the sample DNA **22** reacts with the TPA **17** upon application of the voltage to the metal support **2,** and results luminescence.

[0023] Figures **6** and **7** are diagrams for illustrating the reaction between the ruthenium complex **18** and the TPA **17** on the metal support **2.** First, the TPA **17** discharges an electron on the electrode plate and becomes a cation radical (TPA+*). Since the cation radical is very unstable, it discharges a proton (H+) to be a radical, but because it is still unstable, it further discharges an electron through a reaction with Ru^{3+}. On the other hand Ru^{2+} discharges an electron on the electrode plate to become Ru^{3+} and receive an electron through the reaction with the TPA radial (TPA*), but because it is still unstable (excitation state, Ru^{2+*}) it discharges a photon and returns to stable Ru^{2+}.

[0024] The present invention is not limited to the above-described embodiment.

[0025] The metal support may be, other than the platinum-covered titanium, a platinum plate, a stainless plate, a titanium/platinum clad or the like. The titanium/platinum clad is obtained by mounting a thin platinum plate on a thin titanium plate with bolts. Since the platinum-covered titanium is obtained by plating a titanium substrate with platinum, a rough surface in a molecular level can be obtained as a general outcome of plating. Thus, the platinum-covered titanium is more efficient as an electrode compared to the above-mentioned plati-

num plate, the stainless plate or the titanium/platinum clad. The metal support is a good electric conductor owing to the metal and thus, for example, an insulating substrate such as a glass substrate covered with a metal can be used. The metal is not necessarily exposed on the surface, and the metal surface may be covered with a thin dielectric substance for protecting the metal from the solution, as long as the support functions as a good electric conductor such that a charge can be supplied from the metal support to the solution and that the ruthenium complex can react with the TPA.

INDUSTRIAL APPLICABILITY

[0026] As described above, according to the present invention, a hybridization reaction can be performed in an efficient manner in a short time. By using an electrogenerated chemiluminescent substance for detection, the detection can repeatedly be performed. By adjusting the amount and time of the charge supplied from the electrode, an appropriate luminescent intensity can be obtained for each sample.

Claims

1. A support used for a hybridization reaction, comprising a metal for supplying a charge to a hybridization reaction solution.

2. A support according to claim 1, wherein a biological substance is immobilized on the support.

3. A case used for a hybridization reaction, comprising an electrode for supplying a charge to a hybridization reaction solution.

4. A case according to claim 3, wherein the case accommodates the support of either claim 1 or 2.

5. A hybridization device which supplies electricity to a case used for a hybridization reaction to supply a charge to a hybridization reaction solution.

6. A labeling reagent comprising an electrogenerated chemiluminescent substance for labeling a biological substance.

Fig. 1

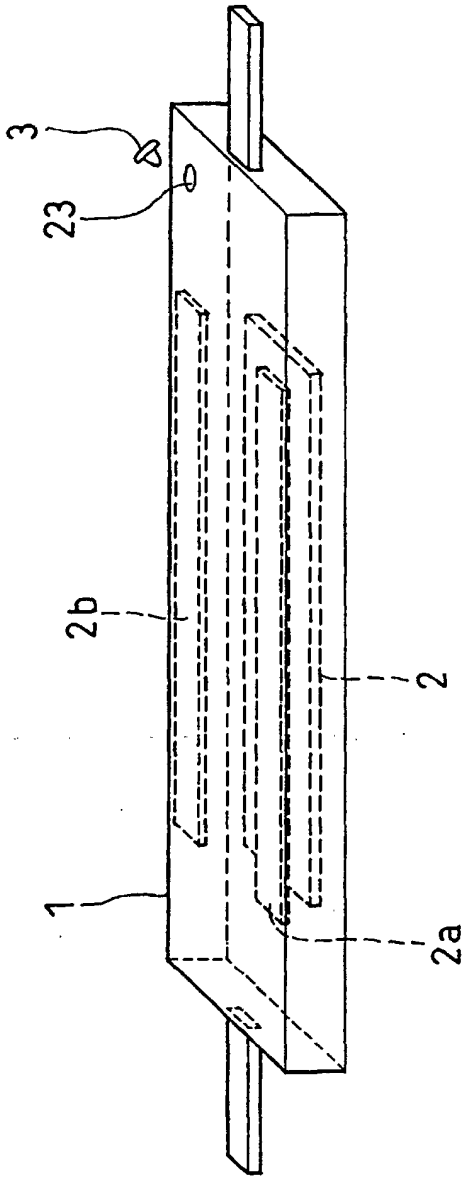


Fig. 2

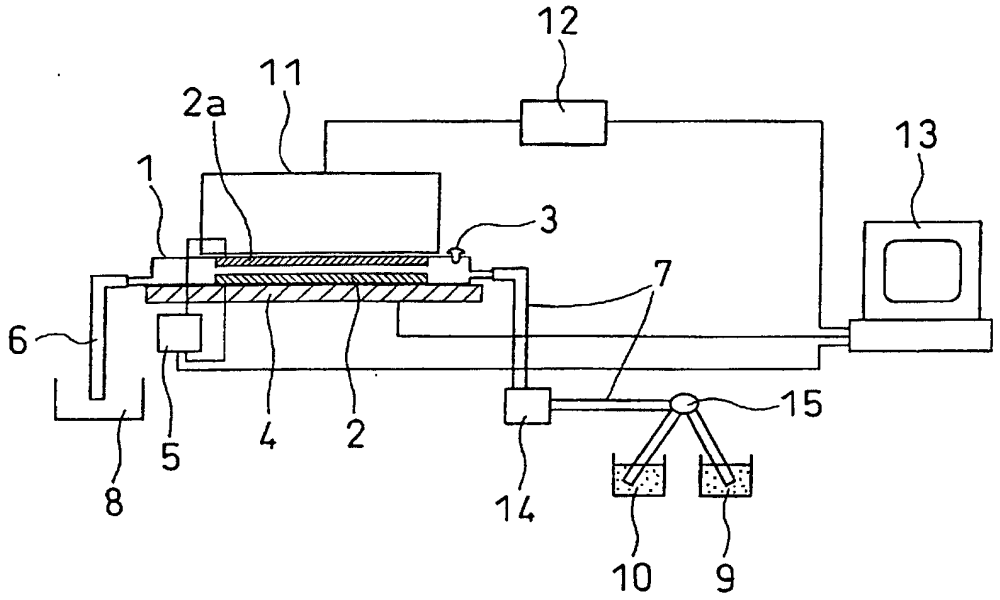


Fig. 3

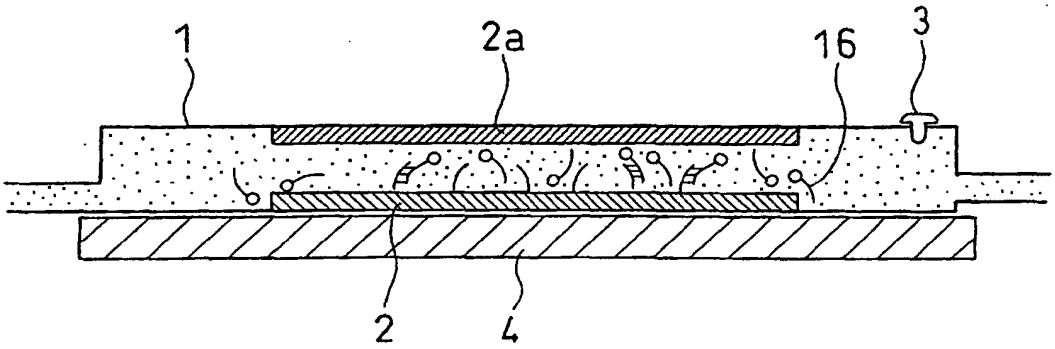


Fig. 4

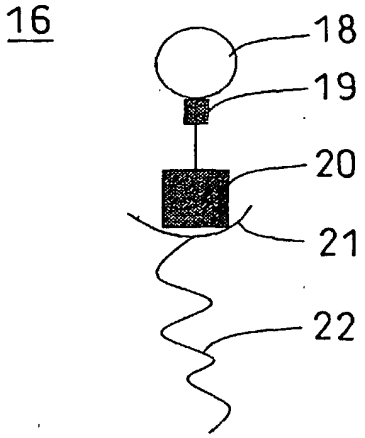


Fig. 5

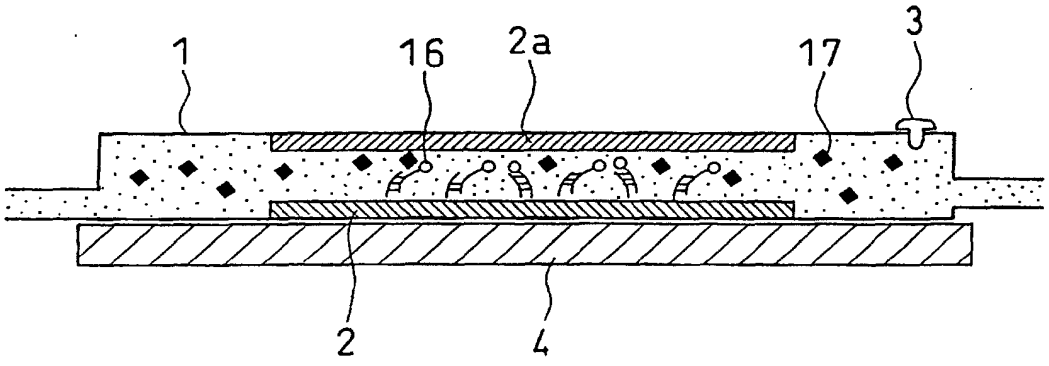


Fig. 6

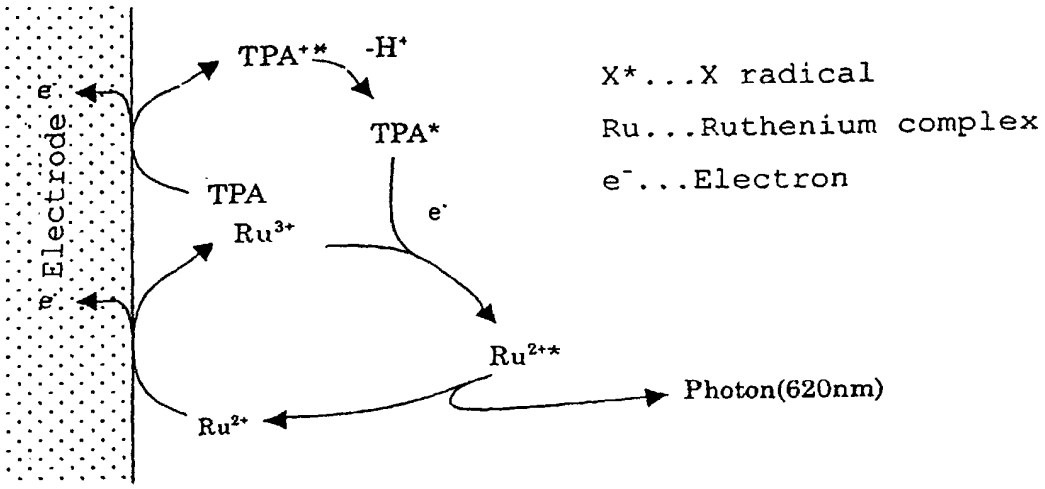
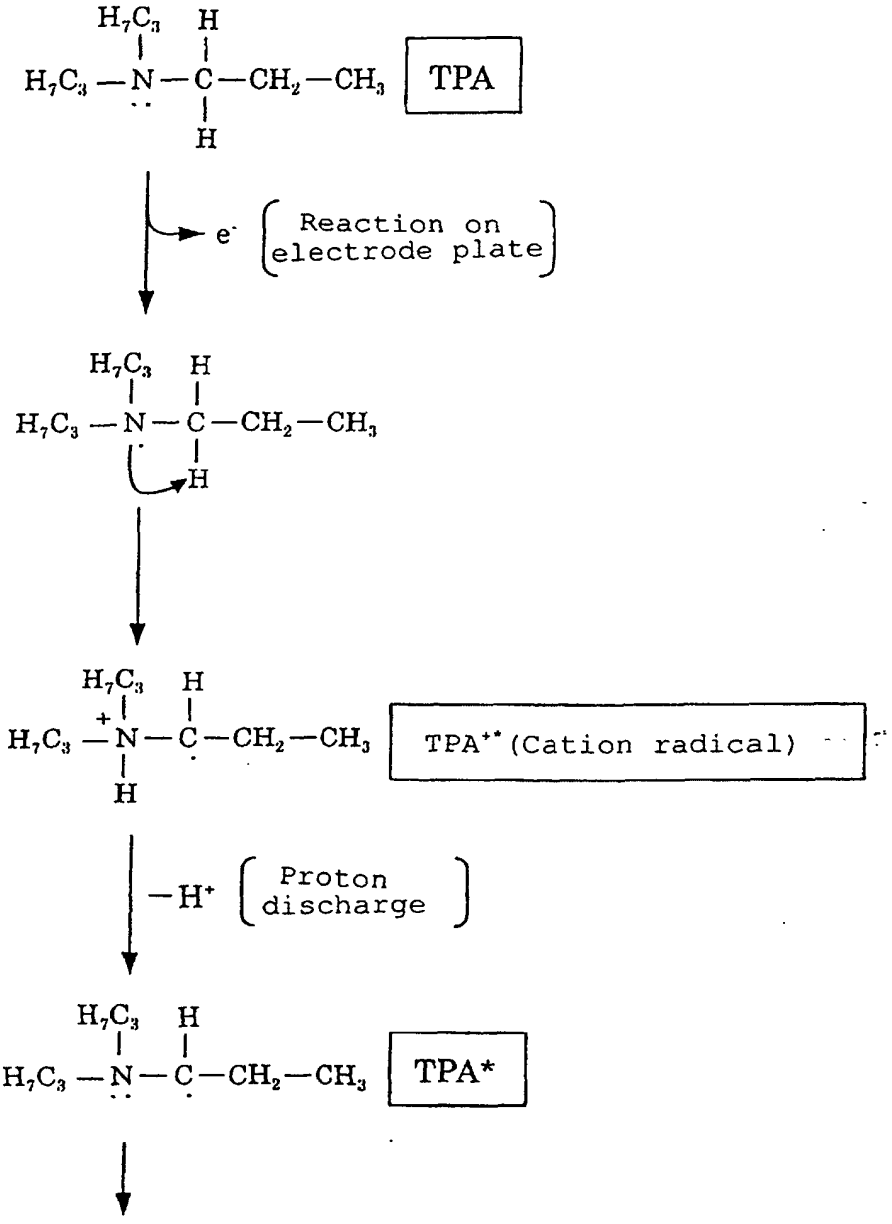

TPA+*
-H+
TPA*
TPA
Ru3+
e-
e- Electrode e-
Ru2+*
Photon(620nm)
Ru2+
X*...X radical
Ru...Ruthenium complex
e-...Electron

Fig. 7


TPA
e⁻
Reaction on electrode plate
TPA⁺• (Cation radical)
−H⁺
Proton discharge
TPA*
Reaction with Ru3+
e⁻ discharge

INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP00/08049

A. CLASSIFICATION OF SUBJECT MATTER
Int.Cl[7] C12M1/00, C12M1/42, G01N33/53, G01N33/566, G01N33/532, G01N21/76, //C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Int.Cl[7] C12M1/00, C12M1/42, G01N33/53, G01N33/566, G01N33/532, G01N21/76, //C12Q1/68

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPI (DIALOG), BIOSIS (DIALOG), JOIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JP, 10-146183, A (TOSHIBA KK), 02 June, 1998 (02.06.98) (Family: none)	1-6
X	JP, 10-239240, A (HITACHI LTD), 11 September, 1998 (11.09.98) (Family: none)	1-6
X	JP, 8-154656, A (NIKON CORP), 08 June, 1996 (08.06.96) (Family: none)	1-6
Y	WO, 93/10267, A (IGEN INT INC, IGEN INC), 27 May, 1993 (27.05.93) & EP, 567635, A & JP, 6-507316, A & US, 5635347, A & AU, 9331412, A & IL, 103754, A	1-6

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 December, 2000 (27.12.00)	16 January, 2001 (16.01.01)
Name and mailing address of the ISA/ Japanese Patent Office	Authorized officer
Facsimile No.	Telephone No.

Form PCT/ISA/210 (second sheet) (July 1992)